UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/661,172	09/13/2003	Jason C. H. Shih	5051-653	7508

20792 7590 10/26/2006

MYERS BIGEL SIBLEY & SAJOVEC
PO BOX 37428
RALEIGH, NC 27627

EXAMINER	
WALICKA, MALGORZATA A	

ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 10/26/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/661,172	SHIH ET AL.	
	Examiner	Art Unit	
	Malgorzata A. Walicka	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *09/22/06 & 09/19/06*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) _____ is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-3,5-13 and 31* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

The Supplemental Amendment after Final and Amendment after Final filed September 22, 2006 and Sept. 19, 2006, respectively, are acknowledged. Both documents contain amendments to claims and to the specification. The Supplemental Amendment of Sep. 22, 2006 presents the sets of claims which replace all prior versions and listings of the claims in the application. For that reason the instant Office Action examines the claims of Sept. 22, 2006. Claims 1-3 and 9-11 have been amended; claim 31 has been added. Claims 4 and 14-30 have been previously cancelled. Thus, claims 1-3, 5-13 and new claim 31 are pending and subject of this Office Action.

After reconsideration of the content of the specification and previous Office actions, the finality of the Office action of June 19, 2006 is withdrawn in favor of the following.

DETAILED ACTION

1. Objections

Specification

The current amendment to page 5 of the specification is objected to as containing new matter. Applicants changed the name B. subtilis DB104 to read B. licheniformis DB104 which is incorrect, because the designation DB104 refers no doubt to B. subtilis; see the article by X. Lin, 1997 (enclosed in IDS) and Wong et al., 2004 (cited by the examiner in 892 Form of 12/29/06). There is nothing as B. licheniformis DB104. Please correct the specification accordingly.

Claims

Objection to clam 1 because it recites the word "collected" is withdrawn, in the light of the amendment.

2. Rejections

2.1. 35 U.S.C. 112, second paragraph

Claim 2 was rejected in the office Action of June 19, 2006 (previous action) as confusing in recitation of the word "substrate". This rejection is withdrawn, because the claim has been amended.

Rejection of claim 11 as being unclear in recitation of "said recombinant Bacillus" is withdrawn, because the claim has been amended.

Claims 1 and 31 are rejected for reciting "a corresponding wild type Bacillus that does not have said at last one kerA coding sequence inserted into the genome thereof" and "a corresponding wild type Bacillus that does not have said at last one Bacillus licheniformis kerA coding sequence inserted into the genome thereof" as unclear. The claims are unclear if the recitations mean a Bacillus cell identical to the recombinant cell claimed except that it lacks any kerA gene ~~et al~~ at all or a Bacillus cell identical to the recombinant cell except that the kerA gene is extrachromosomal and whether Bacillus cell identical to the recombinant cell except that the kerA of Bacillus licheniformis gene is extrachromosomal. For examination purposes it is assumed that wild type B. licheniformis simply contains one copy if its native kerA gene.

2.2. 35 USC section 112, first paragraph

2.2.1. Written description

Claims 1-3 and 5-13 were rejected in the previous action as reciting "heterologous *kerA* gene". This rejection is now withdrawn because the claims have been amended. Rejection of claim 6 for lack of written description of Bacillus kerA gene is withdrawn, because Applicants' arguments have been found persuasive. Claims 1-3 and 5-13 and new claim 31 are rejected under this paragraph.

Firstly, claims 1-3, 5 and 8-13 are rejected as directed to a large and variable genus of methods of using of integrants of *Bacillus licheniformis* and *Bacillus subtilis* species having at least one *kerA* gene inserted into their chromosome. The claims are directed to the use of a large genus of transformants comprising one or several copies of any *kerA* gene. Applicants teach B. licheniformis PWD-1 (Table 4) and B. subtilis DB104 (Table 2) having integrated in at least one copy of *kerA* gene of B. licheniformis. In preparation of their transformants/integrants Applicants used only one kerA gene, which is B. licheniformis kerA. The claims, however, are directed to the integrants having integrated any kerA gene, i.e., to a large genus of integrants comprising a large genus of kerA genes. The only species of kerA genus, i.e., kerA gene of B. licheniformis does not provide an identifying characteristcs of all kerA genes from any organism or man-made. Such genes are encompassed by broad scope of the claims, and the state of art at the time the application was filed does not teach any kerA gene.

For the presented reasons, one of skills in the art is not convinced that Applicants were in possession of the claimed invention at the time the application was filled

Claims 1-3, 5-12 and 31 are rejected for **lack of written description of increase of keratinase production by integrants** of B. licheniformis and B. subtilis containing kerA gene integrated. Applicants have demonstrated that the plant p43 promoter action causes an increase in production of keratinase from Bacillus integrants and not integration of kerA gene itself. In Table 2 integrants of B. subtilis DB104 containing plasmids pNKER1 and pNKER2, both containing keratinase promoter (Pker), have both the same expression of keratinase at 2600 U/ml, whereas integrant containing plasmid pNKER439 comprising both promoters P43 and Pker, has the expression 5200U/ml. This value is only slightly higher than expression by B. subtilis DB104 containing extrachromosomal plasmid pLB29 comprising P43 and Pker. One skilled in the art concludes therefore that the increase in kerA expression in B. subtilis expressing kerA extrachromosomally and intrachromosomally is related to the action of P43 promoter. In case of B. licheniformis, Table 4, integrants having kerA gene under control of keratinase promoter produced less keratinase than the wild type, only addition of the P43 promoter caused an increase in keratinase activity; see strains PTJ1-3 and PTJ6 vs. PWN21, 315, 523, 627, 339.

Furthermore, claim 12 is directed to a large genus of constitutive promoter to be associated with the coding sequences of KerA gene. The genus of constitutive promoters is not sufficiently described in the disclosure, because providing the P43 promoter does not provide the structural characteristics of the whole genus of the

constitutive promoters. The constitutive keratinase promoter does not do the job. Thus, one of skills in the art is not convinced that Applicants were in possession of the claimed invention at the time the application was filled.

2.2.2. Enablement

In the previous action claims 1-3, and 5-13 were rejected for the scope of enablement, because the specification, while being enabling for a method of use of a recombinant *Bacillus subtilis* having at least one heterologous *kerA* gene of *Bacillus licheniformis* inserted into *B. subtilis'* chromosome, does not reasonably provide enablement for B. subtilis or B. licheniformis integrant having any heterologous *kerA* gene inserted into its chromosome. Rejection of claims 6 and 7 is withdrawn, because the specification and prior art enables kerA genes of B. subtilis and B. licheniformis. Rejection of claims 1-3, 5, and 8-13 is maintained for reasons explained in the previous action.

New rejection

Claims 1-3, 5-12 and new claim 31 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim contain subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

As stated above, under paragraph 2.2.1, claims are directed to a large and variable genus of method of making keratinase by integrants of Bacillus licheniformis

and subtilis wherein said integrants produce more keratinase than corresponding wild types and contain in their chromosomes integrated kerA gene. However, due to the lack of written description of integrants having integrated kerA gene and producing more keratinase than the corresponding wild type the claims are directed to the subject matter having scope not disclosed by Applicants.

The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Otherwise, undue experimentation is necessary to make the claimed invention. Factors to be considered in determining whether undue experimentation is required, are summarized *In re* Wands [858 F.2d 731, 8 USPQ 2nd 1400 (Fed. Cir. 1988)]. The Wands factors are: (a) the nature of the invention, (b) the breadth of the claim, (c) the state of the prior art, (d) the relative skill of those in the art, (e) the predictability of the art, (f) the presence or absence of working example, (g) the amount of direction or guidance presented, (h) the quantity of experimentation necessary.

The nature of the claimed invention is a genus of methods using integrants of Bacillus subtilis and Bacilus licheniformis, comprising kerA gene or its multiple copies integrated into their chromosome, for keratinase production that is higher than in wild type.

While methods of engineering microorganisms having a particular gene inserted into their chromosomes are well known in the relevant art and skills of the artisans well developed, no one is able to produce more keratinase by integrants than by the wild types, because the specification teaches that some integrants do not produce more

keratinase than wild type. The production is increased only if the integrated kerA gene is under control of the plant promoter p43; see Table 4. While enablement is not precluded by the necessity for routine screening, if a large amount of screening is required, the specification must provide a reasonable amount of guidance with respect to the direction in which the experimentation should proceed as to make and use the claimed invention. Absent teaching by the claims that the inserted kerA gene has to be under control of p43 promoter the skilled artisan is forced to experimentation that has low probability of success and undue.

2.2. 35 U.S.C. 103

Claims 1, 6, 7 and 9-13 were rejected under 35 U.S.C. 103(a) in the previous action as obvious over Lin et al., (Nucleotide Sequence and Expression of *kerA*, the Gene Encoding a Keratinolytic Protease of *Bacillus licheniforms* PWD-1, Applied and Environmental Microbiology, 1995, 61, 1469-1474, included in the IDS) in view of van der Laan et al. (Cloning, Characterization, and Multiple Chromosomal Integration of a *Bacillus* Alkaline Protease Gene, Applied and Environmental Microbiology, 1991, 57, 901-909, included in the IDS) and the product of the Dutch Firm DSM, which is integrative plasmid pLAT8 specific for *Bacillus*.

Rejection of claims 1, 6, 7 and 9-12 and 13 are also rejected. Claims 1, 6, 7, 9-11 are directed to a method of making a keratinase, comprising:

a) culturing a recombinant Bacillus in a medium, said recombinant Bacillus

selected from the group consisting of Bacillus licheniformis and Bacillus subtilis and having at least one kerA coding sequence inserted into the chromosome thereof, with said recombinant Bacillus producing greater quantities of keratinase than a corresponding wild type bacillus that does not have said at least one kerA coding sequence inserted into the genome thereof

(b) isolating said keratinase form medium,

i.e. the claims are not limited to the integrants that expressed kerA gene under control of their native promoters, i.e. they may have not native promoters as part of the integrated DNA construct.

Claims 12 and 13 are directed to a method of making keratinase comprising:

(a) culturing a recombinant Bacillus in a medium, said recombinant Bacillus selected from the group consisting of Bacillus licheniformis and Bacillus subtilis and having at least one kerA coding sequence inserted into the chromosome thereof, with said recombinant Bacillus producing greater quantities of keratinase than a corresponding wild type bacillus that does not have said at least one kerA coding sequence inserted into the genome thereof, wherein said kerA coding sequence is operatively associated with a constitutive promoter or p43 promoter; and then

(b) isolating said keratinase form medium.

Lin et al. teach the encoding DNA and amino acid sequence of keratinase (serine protease) from *B. licheniformis*. Lin et al. teach high expression of keratinase under control of p43 promoter in S. subtilis DB104 transformed with plasmids remaining in B.

subtilis extrachromosomally, however, Lin does not teach the production of said keratinase in integrants of *Bacillus*.

Van der Laan et al. teach that efficient expression of a serine protease may be achieved in recombinants of *Bacillus* cells wherein said recombinants have the gene of the protease integrated into their chromosomes. Van der Laan also teaches the integrants are more stable than transformants possessing extrachromosomal expression vectors; see the abstract of the article. See also page 905, left column, subtitle "Production improvement of the alkaline serine protease of strain PB92", where the authors describe production of serine protease of *Bacillus alcalophilus* **in *Bacillus subtillis.*** The Bacillus subtilis was transformed by Laan et al. using a protoplast method ensuring a stable integration of the protease gene. The Dutch firm DSM produces pLAT8 plasmid (containing alpha–amylase gene of *Bacillus)* which is used by scientific community for integration of DNA into the chromosome of *Bacillus.*

It would have been obvious for one having ordinary skills in the art to have *kerA* gene of Lin et al. under control of p43 promoter and express it by integration to a chromosome of *B. subtilis as* van der Laan et al. did, using a commercially available integration plasmid pLAT8. The motivation would have been to obtain a cell stably engineered to produce large quantities of keratinase. The motivation is taught by van der Laan et al. who emphasize that their methods gives the stable transformants for production of Bacillus serine protease; see the abstract and the end of introduction. Keratinase is a Bacillus serine protease. The expectation of success is high, because

van der Laan proved the successful production of Bacillus serine protease by integrants of *Bacillus subtilis*.

Therefore, the invention claimed in claim 13 was within the ordinary skill in the art to make and use at the time it was made, and was as a whole, *prima facie* obvious.

Response to Applicants arguments

Applicants argue in their REMARKS, page 9 of 19, second paragraph that van der Laan et al., is directed to the cloning, characterization and multiple chromosomal integration of Bacillus high-alkaline protease gene from Bacillus alcalophilus, thus the requisite motivaton to combine the cited references is lacking.

This argument of Applicants is found not persuasive, because one having skills in the art realizes that a method of expression of one Bacillus serine protease in integrants of Bacillus subtilis is suitable for expression another Bacillus serine protease in integrants of Bacillus subtilis, absent teachings to the contrary, i.e., absent teaching by Laan et al. that the method is applicable to high-alkaline protease of Bacillus alcalophilus exclusively. Laan et al. teach integration of a Bacillus serine protease gene into the chromosome of Bacillus subtilis and its expression therefrom. In the instant application Bacillus serine protease (B. licheniformis keratinase) is integrated into B. subtilis chromosome and expressed therefrom.

3. Conclusion

All claims are rejected.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Malgorzata A. Walicka whose telephone number is (571) 272-0944. The examiner can normally be reached on Monday-Friday from 10:00 a.m. to 4:30 p.m.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapura Achutamurthy, can be reached on (571) 272-0928. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Malgorzata A. Walicka, Ph.D.
Art Unit 1652
Patent Examiner

REBECCA E. PRUTY
PRIMARY EXAMINER
GROUP ~~1800~~ 1600